new minus-ends and begins pulling them poleward. These pole-focusing forces exist throughout metaphase and anaphase and can move chromosomes rapidly, dominating other spindle forces. Opposing forces on chromosomes from the other half-spindle are able to slow, though not stop, the pole-focusing response, as indicated by faster pole-focusing speeds in monopolar spindles and during anaphase than in metaphase bipolar spindles. Together, our data indicate that microtubule minus-end focusing forces operate broadly and rapidly and are of similar magnitude to other spindle forces. These pole-focusing forces are thus well-suited to robustly maintain spindle structural integrity despite rapid turnover of spindle components and mechanical challenges.

64-Subg
Structural, Mechanical, and Biochemical Insights into the Mechanism of Myosin Force Sensing
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Myosin-Is are widely expressed molecular motors that comprise the second largest myosin family in vertebrates with eight isoforms. Myosin-Is participate in a host of cellular processes including vesicular trafficking, membrane dynamics, and nuclear transcription. The question thus arises, how can these motors with similar biochemical properties give rise to such diversity of function? The answer appears to lie in the biochemical and mechanical diversity of the isoforms. Our biophysical studies demonstrate that Myo1b is exquisitely sensitive to tension, where forces >0.5 pN cause the motor to transform from a low duty ratio motor with attachment lifetimes <1 s to a high duty ratio motor with attachment lifetimes >50 s. Our studies also reveal that the isoform Myo1c has a very different response to force despite its similar unloaded kinetics to Myo1b. Myo1c is far less sensitive to force than Myo1b, enabling it to power motility over a range of forces, consistent with it serving a role as a transporter rather than as a tension-sensitive anchor. To better understand the molecular basis for these differences in force sensing, we have determined the crystal structure of Myo1b's motor domain and first IQ-motif with bound calmodulin. The structure reveals novel interactions between the light-chain binding domain and the N-terminus of the motor domain, a region that shows substantial sequence variability among myosin-I isoforms. We propose that these interactions facilitate communication between the lever arm and the ATP binding site, modulating the chemomechanical properties of the motor. Supported by the NIH (GM057247).

65-Subg
A Structural Model of the Kinesin-5 Mechanochemical Cycle
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Kinesin motor domains drive diverse microtubule-based, ATP-dependent activities but the molecular adaptations that specify these diverse functions are poorly understood. Kinesin-5s are essential mitotic motors and their inhibition with specific small molecules blocks cell division. Using cryo-electron microscopy and subnanometer resolution structure determination, we have visualised conformations of microtubule-bound human kinesin-5 motor domain at successive steps in its ATPase cycle. In the ATP-like state, the kinesin-5 neck-linker is directed towards the microtubule plus-end, consistent with its role in directional force generation. As ATP hydrolysis proceeds, nucleotide-dependent conformational changes in the active site are allosterically propagated into rotations of the motor domain, uncurling of the drug-binding loop5 and discrete, ratchet-like displacements of the neck linker that contribute to motor stepping. The motor N-terminus also undergoes large reorientations that indicate its role in controlling kinesin-5 neck-linker conformation throughout the motor's ATPase cycle. A kinesin-5 mutant lacking this N-terminus is enzymatically active, but ATP-dependent neck linker movement and motility is defective, although not totally ablated. Our data demonstrate that, while the motor N-terminus plays a kinetic role in controlling efficient neck-linker movement, the kinesin-5 neck-linker has intrinsic biophysical properties that enable it undergo nucleotide-dependent ratchet-like movements that have presumably evolved according to specific functional requirements.

66-Subg
From Extensile Microtubules Bundles to Synthetic Cilia and Self-Mixing Active Gels
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In presence of a macromolecular crowding agent filamentous microtubules spontaneously assemble into elongated bundles. Kinesin clusters can simultaneously bind to multiple filaments within such a bundle and thus power relative sliding of the constituent filaments and the overall bundle extension. Starting with such extensile bundles it is possible to hierarchically assemble diverse biologically inspired materials including spontaneously beating synthetic cilia, self-mixing and self-flowing active gels, motile emulsion droplets as well as deformable vesicles. However, little is known about the mechanical properties of isolated active extensile bundles that are the essential structural motif of these diverse materials. We describe an experimental technique that allows us to systematically assemble filamentous bundles with predetermined number of filaments and quantify its ability to generate extensile force.

67-Subg
Tug-of-War: Mechanical Coordination of Molecular Motors
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Molecular motors often perform their functions in small teams rather than as individual molecules. An important issue for understanding the function of motor teams is how the motors are coordinated. It has become clear in recent years that mechanical interactions between motors play an important role in such processes, as motors exert forces on each other. I will discuss two cases, where experimental evidence has recently been obtains for the presence of such mechanical interactions: bidirectional cargo transport by cytoskeletal motors of opposite polarity (e.g. kinesins and dyneins) and the twitching motility of bacteria on surfaces powered by pilus motors. Stochastic tug-of-war models explain how fast bidirectional motion (or persistent motion in random direction) is obtained despite the presence of opposing forces via an instability caused by the forced unbinding of motors. It will be shown that the mechanical interactions alone are sufficient to account for the experimentally observed dynamics.

68-Subg
Mechanisms of Dynein-Driven Microtubule Sliding and Cargo Transport
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Dynein is a minus-end-directed microtubule motor protein that powers the beating of cilia and flagella and transports a wide variety of cargoes within the cytoplasm of eukaryotic cells. Dynein is not evolutionarily related to the cytoskeletal motor proteins kinesin and myosin, but instead is a member of the AAA+ superfamily. Unlike most AAA+ ATPases that self-assemble into homo-hexameric rings, dynein has six distinct AAA+ domains that are concatenated within a single, large polypeptide chain; extending from one of the AAA+ domains is a long, anti-parallel coiled-coil extension (called the stalk) that binds to microtubules. We have studied the mechanism of dynein motility using a combination of structural and single molecule studies. I will discuss how the two motor domains of dynein can act to slide apart anti-parallel microtubules, an activity that may be important for dynein's actions in organizing the mitotic spindle during mitosis. I also will discuss how dynein's enzymatic cycle might be regulated for normal cargo transport.

Subgroup: Exocytosis & Endocytosis

69-Subg
Exocytotic Fusion Pore Intermediates of Dense-Core Vesicles
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Regulated exocytosis is a multistage process involving a merger between the vesicle and the plasma membranes, leading to the formation of a fusion pore, a channel, through which secretions are released from the vesicle to the cell exterior. A stimulus may influence the pore by either dilating it completely (full-fusion exocytosis) or mediating a reversible closure (transient exocytosis). In neurons, these transitions are short-lived and not accessible for